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Intralesionally Implanted Cisplatin Plus Systemic Carmustine for the Treatment of Brain Tumor in Rats

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Background and Objectives: The benefit of conventional chemotherapy for the treatment of malignant brain tumors, although limited, is real. A major obstacle in the treatment of these lesions is the ability to deliver drug across the blood-brain barrier (BBB). Local drug implantation, circumventing the BBB, has been a useful strategy for treatment of intracranial lesions, and may work synergistically with systemic chemotherapy. To test this hypothesis, either intraperitoneal (ip) carmustine or cisplatin was combined with the intracranial (ic) administration of polymer-delivered cisplatin in rats with intracranial tumors.

Methods and Results: 9L gliosarcoma tumor cells (5×10^3) were administered through a right frontal lobe cannula in rats 7 days prior to treatments. Cisplatin-loaded biodegradable polymer was then administered via the cannula, with free cisplatin or carmustine injected ip. Animals were monitored for 60 days post-treatment. In experiment 1, ic cisplatin at a dose of 0.5, 1.0, 2.0, and 4.0 mg/m^2 resulted in a mean survival time of 34 ± 3, 39 ± 14, 47 ± 11, and 31 ± 20 days (MST ± SD), respectively, compared to 26 ± 4 days in the control group and 30 ± 7 days in the group treated with 50 mg/m^2 ip free cisplatin. In experiment 2, ip free cisplatin at 25, 40, 50, and 100 mg/m^2 resulted in a MST of 28 ± 3, 30 ± 4, 32 ± 3, and 14 ± 8 days, respectively, compared to 26 ± 1 days in the control group. In experiment 3, the MST in the groups treated with 0.5 mg/m^2 of ic cisplatin, 25 mg/m^2 of ip cisplatin, 10 mg/kg of ip carmustine, ic cisplatin (0.5 mg/m^2) plus ip cisplatin (25 mg/m^2), and ic cisplatin (0.5 mg/m^2) plus ip carmustine (10 mg/kg) was 30 ± 4 days ($P > 0.05$), 28 ± 2 ($P > 0.05$), 36 ± 4 ($P < 0.01$), 32 ± 3 ($P < 0.01$), and 50 ± 11 days ($P < 0.01$), respectively, compared to the tumor control group (26 ± 1 days). Long-term survivors (29%) were seen only in the ic cisplatin plus ip carmustine group. Additive toxicity was not observed.

Conclusions: Intralesional polymer-delivered (ic) cisplatin plus systemic (ip) carmustine is highly effective for the treatment of intracranial 9L gliosarcoma in tumors. *J. Surg. Oncol. 1998;69:76–82.*

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INTRODUCTION

The benefit of conventional chemotherapy for the treatment of intracranial (ic) tumors, although limited, is real [1,2]. A major obstacle in the treatment of these lesions is the delivery of drug across the blood-brain barrier (BBB). Local drug implantation has been useful in overcoming the BBB, and has been shown to increase survival in animals with ic gliomas [1–4] and in humans with recurrent malignant gliomas [5,6]. The optimum therapeutic dose, however, has yet to be determined and appears dependent on tumor size [7]. Previous studies have shown that for 1 week established ic 9L gliosarcomas in rats, doses of cisplatin equal to or greater than 5 mg/m^2 induced significant toxicity in normal brain [1,2]. Doses less than 0.5 mg/m^2 demonstrated negligible toxicity, but resulted in a survival that was not significantly prolonged over control animals.

Tumors in general exhibit a considerable diversity in regional blood flow [8] and local intratumoral metabolic activity [9]. Therefore, individual tumor cells may vary in their response to particular cytotoxic agents [10]. Considering this heterogeneous response of tumors to chemotherapy and the variable trafficking of these therapeutic agents through brain tissue depending on the route of administration, we proposed a chemotherapeutic strategy in the treatment of brain tumors combining different chemotherapeutic agents, each with its own unique mechanism of toxicity, administered via different routes.

Chemotherapeutic agents that are lipid soluble have a greater ability than hydrophilic agents to penetrate the BBB when administered intravenously (iv). Carmustine, a lipid-soluble chemotherapeutic agent, is currently the most effective drug used systemically for the treatment of primary ic tumors. In comparison, cisplatin, a hydrophilic agent, has less ability to penetrate the BBB [11,12] when administered systemically, but has been shown to have a higher affinity to concentrate within brain tumor tissue [13].

Based on these considerations, we hypothesized that the combination of ic cisplatin and systemic carmustine would act synergistically through the delivery of the hydrophilic cisplatin to the center of the tumor, combined with a constant level of systemically delivered carmustine to the periphery. This treatment strategy was expected to improve the overall therapeutic efficacy of each agent alone, as well as to decrease potential drug resistance.

MATERIALS AND METHODS

Experimental Design

Previous work in our laboratory had shown that doses of cisplatin equal to or greater than 5 mg/m^2 induced significant toxicity in normal brain [2,3]. Doses less than 0.5 mg/m^2 demonstrated negligible toxicity, but resulted in a survival that was not significantly improved over control animals. In addition, the optimum therapeutic dose appeared to be dependent mainly on tumor size or tumor stage, and the presence of ic 9L gliosarcomas had buffering ability against the side effects of chemotherapy [7]. The clinically applied optimum therapeutic dose theoretically should be greatly individualized. Based on these considerations, the major purpose of the present study is to test the advantages of the therapeutic strategy instead of the optimum therapeutic dose. However, in order to avoid an unexpected added toxicity following the combined treatments, the escalating doses of cisplatin at 0.5, 1.0, 2.0, and 4.0 mg/m^2 in polymer, and free cisplatin at 25, 40, 50, and 100 mg/m^2 were tested in experiment 1 and experiment 2, respectively. These two experiments were designed to explore suboptimum doses. In experiment 1, the ic cisplatin in polymer was also compared to the free ip cisplatin at 50 mg/m^2. The dose of carmustine used in this study was 10 mg/kg, which was determined based on our previous experiment (data not shown). In experiment 3, the suboptimum dose of cisplatin in polymer was administered ic, combining the suboptimum doses of free cisplatin or carmustine administered ip. Tumor cells were administered through a right frontal lobe cannula in rats 7 days prior to treatments. Long-term survivors were sacrificed 60 days after tumor inoculation.

Tumor Cells

A low passage number of 9L gliosarcoma cells, isolated from Fischer 344 rats, were cultured in Dulbecco's modified Eagle's medium containing 15% fetal calf serum (Gibco, Grand Island, NY), 200 mM L-glutamine, 100 U/ml penicillin, and 0.1 mg/ml streptomycin for 4–5 days. Cell were then washed and resuspended in phosphate-buffered saline before use.

Procedure of Cannulation

Inbred pathogen-free male Fischer 344 rats weighing between 200 and 250 g were used (SASCO, Omaha, NE). The animal use protocol was reviewed and approved by the Institute of Animal Care and Use Committee, University of Colorado Health Sciences Center, following the Guide for the Care and Use of Laboratory Animals as established by the Institute of Laboratory Animal Resources Commission on Life Sciences of the National Research Council. The detailed cannulation procedure has been described elsewhere [2,4]. Rats were anesthetized with a combination of ketamine (44 mg/kg)

and xylazine (8 mg/kg) administered intramuscularly (im). A stainless steel cannula with a 0.065 in. outside diameter (O.D.) and 0.053 in. inside diameter (I.D.) (Small Parts, Miami, FL) was stereotactically placed into the right frontal lobe at a point 2 mm anterior and 3 mm lateral to the bregma. The cannula was temporarily occluded with a sterile stylet (0.050 in. O.D.). The animal was allowed to recover from the surgical procedure for 7 days before further manipulation.

Infusion of Tumor Cells

Ic infusion of the tumor cells was performed with unanesthetized rats wrapped in toweling with only their heads exposed. To infuse the cells, the stylet was removed from the cannula. Phosphate-buffered saline (10 μl) containing 5,000 9L gliosarcoma tumor cells was drawn up into a flexible Teflon tubing (0.028 in. O.D. × 0.022 in. I.D.; Small Parts) attached to a 50 μl Hamilton syringe. The cells were then infused into the brain through the cannula over a 2 min period. When finished, the sterile stylet was placed back into the cannula and the animal returned to its cage.

Administration of (Ip) Carmustine, Cisplatin, and (Ic) OPLA®-Pt

Cisplatin-impregnated biodegradable polymer, Open Cell Polylactic Acid Polymer (OPLA®-Pt), was supplied in a concentration of 8.2% cisplatin by weight. OPLA® is the registered trademark of THM Biomedical, Inc. (Duluth, MN), with the internal architecture previously described [14]. The ic administration of polymer was performed with unanesthetized rats wrapped in toweling with only their heads exposed. Preweighed doses of polymer were introduced into the cannula using a specifically designed plastic cone. The polymer was then injected into the brain by use of a small plunger. When finished, the stylet was placed back into the cannula. Carmustine and cisplatin were injected intraperitoneally (ip) with a 25 gauge needle. Approximately 1–3 ml of solution was injected directly into the peritoneal cavity using a 3 cc syringe.

Histological Study

At the time of death, the brain was harvested and fixed in 10% formalin for 7 days. Once fixed, the brain was sectioned coronally at 6 μm intervals, spanning the area of tumor inoculation. Each section was then mounted and stained with hematoxylin-eosin (H&E).

Statistical Analysis

Mean survival time (MST) between treatment groups was compared using the Student *t*-test and log-rank analysis. *P*-values were considered significant when <0.05 and very significant when <0.01.

RESULTS

Determination of the Optimum Ic Dose of Cisplatin

Cisplatin at 0.5, 1.0, and 2.0 mg/m^2 in polymer resulted in a MST of 34 ± 3, 39 ± 14, and 47 ± 11 days, respectively. In all groups, survival was significantly increased ($P = 0.0004$, 0.04, and 0.002, respectively) compared to the tumor control (MST = 26 ± 4 days) (Fig. 1, Table I). The group treated with 2.0 mg/m^2 ic cisplatin in polymer (group 4) demonstrated the longest survival, with 29% of animals surviving to day 60. All these animals demonstrated no evidence of viable ic tumor when examined histologically. Cisplatin at 4.0 mg/m^2 ic (group 5) resulted in a MST of 31 ± 20 days, which was not significantly different from the control. This increased toxicity at the higher cisplatin dose is similar to what we have reported previously [1–3]. The MST of rats treated with ip free cisplatin at 50 mg/m^2 was 30 ± 7 days. Therefore, cisplatin at 2.0 mg/m^2 in polymer was demonstrated to be the optimum dose for the treatment of rats with 1 week established ic 9L gliosarcoma, being more effective than the ip administration of cisplatin at 50 mg/m^2.

Optimum Dose of Free Systemic (Ip) Cisplatin

In this experiment, free cisplatin at 25, 40, 50, and 100 mg/m^2 was injected ip (Table II, Fig. 2). MST in animals treated at 40 mg/m^2 (group 3) and 50 mg/m^2 (group 4) was significantly prolonged over the tumor control (MST = 30 ± 4, 32 ± 3, and 27 ± 3 days, respectively). The *P*-value was 0.02 and 0.0003, respectively. When ip cisplatin was increased to 100 mg/m^2 (group 5), 10 of 11 animals died in 5 days, with the MST significantly shorter than all other treatment groups, as well as the tumor control ($P < 0.0001$).

Synergistic Effect of Intralesionally (Ic) Polymer-Delivered Cisplatin With Systemic Free (Ip) Cisplatin or Carmustine for the Treatment of Rats With 1 Week Established 9L Gliosarcoma

Based on the results obtained in the previous experiments, ic OPLA®-Pt at 0.5 mg/m^2 (which was tolerable in normal rats [2]), ip cisplatin at 25 mg/m^2, and ip carmustine at 10 mg/kg were chosen to test any added benefits in the addition of systemic carmustine or cisplatin to the ic released cisplatin. The lowered doses of cisplatin, instead of the optimal ones, were chosen to avoid additive toxicity. In this experiment (Table III, Fig. 3), the MST was 26 ± 1 days in the tumor control group (group 1), 30 ± 4 days in the ic OPLA®-Pt-treated group (group 2), and 28 ± 2 days in the ip cisplatin-treated group (group 3). Survival in these latter two groups was not significantly prolonged over the control ($P > 0.05$). The combination of ip cisplatin and ic OPLA®-Pt (group 5), however, resulted in a MST of 32 ± 3 days, which was

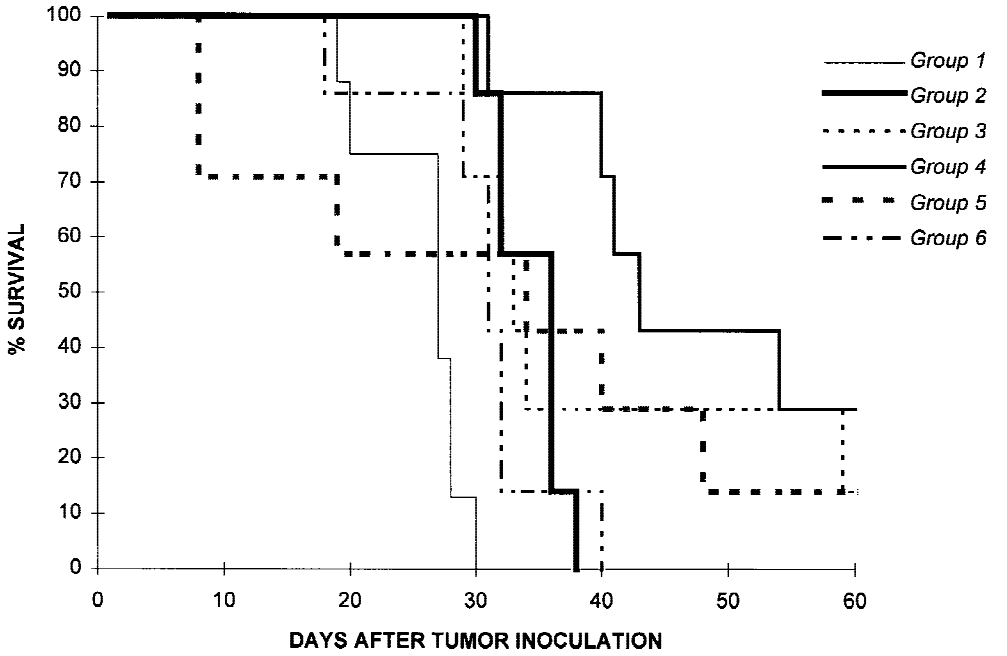

Fig. 1. Kaplan-Meier survival curves demonstrate survival of rats with 1 week established brain 9L gliosarcoma following ic implantation of cisplatin-loaded biodegradable polymer (OPLA®-Pt). Group 1, tumor control; group 2, treated with OPLA®-Pt at 0.5 mg/m^2; group 3, 1.0 mg/m^2; group 4, 2.0 mg/m^2; group 5, 4.0 mg/m^2; group 6, treated with ip cisplatin (50 mg/m^2) (see Table I).

TABLE I. Intralesional OPLA®-Pt for the Treatment of Rats With 1 Week Established 9L Gliosarcoma

Group (N)	Ic OPLA®-Pt (mg/m^2)	Ip cisplatin (mg/m^2)	MST ± SD (days)
1 (8)	–	–	26 ± 4
2 (7)	0.5	–	34 ± 3
3 (7)	1	–	39 ± 14
4 (7)	2	–	47 ± 11
5 (7)	4	–	31 ± 20
6 (7)	–	50	30 ± 7

TABLE II. Rats With 1 Week Established 9L Gliosarcoma Treated by Ip Cisplatin

Group (N)	Ip cisplatin (mg/m^2)	MST ± SD (days)
1 (12)	–	27 ± 3
2 (10)	25	28 ± 3
3 (10)	40	30 ± 4
4 (12)	50	32 ± 3
5 (11)	100	14 ± 8

a significant improvement over both the tumor control group ($P = 0.006$) and the ip cisplatin alone group ($P = 0.025$). MST (36 ± 4 days) in the ip carmustine-treated group (group 4) was significantly longer than that in the tumor control ($P = 0.002$). When ic OPLA®-Pt was added to ip carmustine (group 6), the MST (50 ± 11 days) was significantly improved not only over that of the ic OPLA®-Pt alone group ($P = 0.01$) (group 2) and the ip carmustine group ($P = 0.04$) (group 4), but also over the

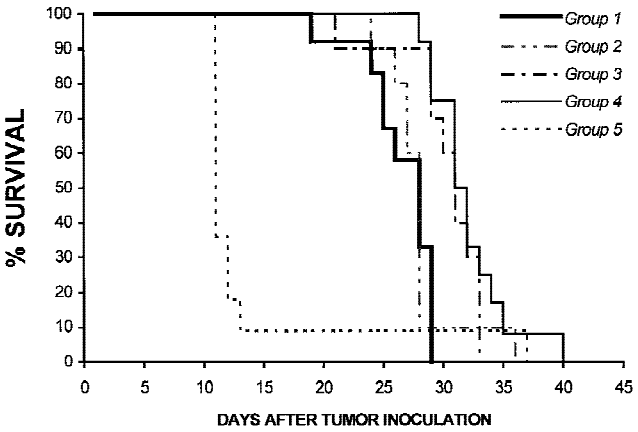

Fig. 2. Kaplan-Meier survival curves demonstrate survival of rats with 1 week established brain 9L gliosarcoma following ip cisplatin. Group 1, tumor control; groups 2–5, treated with ip cisplatin at 25, 40, 50, and 100 mg/m^2, respectively (see Table II).

combination of ic OPLA®-Pt and ip cisplatin ($P = 0.007$) (group 5). Complete histologic eradication of tumor was seen only in the group treated with the combination of ic OPLA®-Pt and ip carmustine (29%). No significant side effects were observed in any of the groups tested. The combined use of ip carmustine and ic cisplatin in polymer was the most effective strategy tested.

DISCUSSION

The efficacy of conventional chemotherapy in the treatment of solid tumors has been limited by the ability

TABLE III. Intralesional OPLA®-Pt and Ip Cisplatin or Carmustine for the Treatment of Rats With 1 Week Established 9L Gliosarcoma

Group (N)	Ic OPLA®-Pt 0.5 mg/m^2	Ip cisplatin 25 mg/m^2	Ip carmustine 10 mg/kg	MST ± SD (days)
1 (6)	−	−	−	26 ± 1
2 (6)	+	−	−	30 ± 4
3 (6)	−	+	−	28 ± 2
4 (6)	−	−	+	36 ± 4
5 (6)	+	+	−	32 ± 3
6 (6)	+	−	+	50 ± 11

of these drugs to reach their targets in adequate quantities. The study of the molecular and cellular biology of individual neoplastic cells has failed to explain the nonuniform uptake of systemically administered drugs, since a solid tumor in vivo is more than a collection of individual cancer cells. A therapeutic agent, once injected into the bloodstream, reaches its target tumor cells through distribution in the blood, movement across the blood vessel wall, and diffusion within the interstitial space. Therefore, the extracellular compartments, including vascular and interstitial, may be the real barriers to the delivery of blood-borne agents.

The first compartment, the blood supply, is generally very heterogeneous in solid tumors [15–18]. This heterogeneity limits the delivery of blood-borne agents to poorly perfused regions of a tumor. The second compartment, the interstitial space, is usually under high fluid pressure within a tumor [19–21], thereby reducing the extravasation of fluid and drug. This increased interstitial fluid pressure in turn leads to an outward pressure gradient at the tumor periphery which opposes inward diffusion [15,16]. These factors taken together account for a third factor, a slow rate of diffusion in the interstitial space, leading to low drug concentrations in the distal regions of a tumor. Finally, the findings of intermittent blood flow in the tumor microvasculature [22] (believed to contribute to heterogeneity in tumor oxygen delivery) and transient vessel non-perfusion [22] (thought to result in acutely hypoxic cells resistant to conventional radiotherapy) are added obstacles in the optimum delivery of conventional chemotherapy. Due to these micro- and macroscopic heterogeneities in tumors, the relative magnitude of each of the above physiological barriers varies from one location to another and from one time period to another [15,16]. For brain tumors, the presence of the BBB will make drug delivery more complicated and difficult.

For the treatment of primary ic gliomas, local drug implantation has been demonstrated to be more effective than the systemic administration of chemotherapy [2,23]. The advantages of drug delivery via biodegradable polymer have been described previously [2,4]. Locally delivered chemotherapy, however, may have difficulty in controlling tumor cells located at the tumor periphery, particularly tumor cells located at some distance from the primary tumor mass. These residual tumor cells are usually the major source of local tumor recurrence [24] with the exact extent of chemotherapy diffusion from the initial implantation site still in question [25].

Based on the above considerations, intralesional sustained drug release through the use of biodegradable polymer is hypothesized to be synergistic with systemic chemotherapy. Theoretically, this strategy is expected to deliver a high drug concentration to the tumor center (by local drug implantation) and a constant level to the periphery (through systemic administration). Ideally, the drug implanted intralesionally should have a poor permeability through the BBB to prevent escape [26,27], with the systemically administered drug having a relatively high permeability through the BBB. As the permeability of cisplatin through the BBB is poor and the permeability of carmustine is relatively high, cisplatin implanted ic in polymer plus carmustine administered systemically theoretically makes good sense. Additionally, a given tumor may prove to be resistant to a given chemotherapeutic agent or the tumor may develop resistance over the course of treatment. This resistance, whether natural or induced, in most instances cannot be overcome by simply increasing the dose. Using two agents, such as carmustine and cisplatin, which kill cells via different mechanisms, may lessen the tumor's ability to develop this drug resistance [28].

Our results demonstrate that the administration of ic cisplatin via polymer was more effective than its administration systemically, which is consistent with our previous reports [2]. As for ip administration, we showed that carmustine, the most commonly used agent clinically [29], was more effective than ip cisplatin. We also demonstrated that the administration of ic cisplatin in polymer was synergistic with either ip cisplatin or ip carmustine. Finally, the combination of ip carmustine and ic cisplatin resulted in a significantly prolonged mean survival time (MST), even compared to combined ic and ip cisplatin. Complete tumor eradication was seen only in the group receiving ip carmustine and ic cisplatin (29%). Of note, side effects did not appear to be additive. Clearly, this new chemotherapeutic strategy, combining intralesional sustained release cisplatin and ip carmustine is reasonable and promising. Studies testing the therapeutic advantages of such combination, using optimum doses, are under way.

In summary, the administration of systemic carmustine in combination with intralesional sustained release cisplatin from a biodegradable polymer is a highly effective strategy for the treatment of intracranial 9L gliosarcoma. Based on these findings, the use of combined therapy in the treatment of primary high grade gliomas in humans seems feasible.

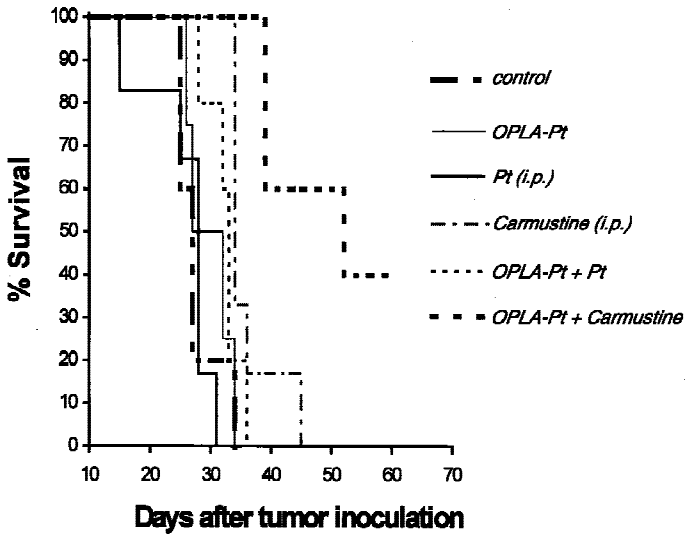

Fig. 3. Kaplan-Meier survival curves demonstrate survival of rats with 1 week established brain 9L gliosarcoma following ic implantation of cisplatin (OPLA®-Pt) at 0.5 mg/m^2, ip cisplatin at 25 mg/m^2, ip carmustine at 10 mg/kg, and their combinations (see Table III).

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